75.035

Accuracy of an 'in-house' sputum polymerase chain reaction assay for rapid diagnosis of sputum smear negative pulmonary tuberculosis at Mulago hospital, Uganda

L. Nakiyingi[1,*], P. Ocama[1], B. Asiimwe[2], F. Katabazi[2], A. Katamba[3], M. Joloba[2], H. Mayanja-Kizza[3]

[1] *Infectious diseases Institute, Makerere University College of Health Sciences, Mulago Hospital Complex, Kampala, Uganda*
[2] *Makerere University College of Health Sciences, Kampala, Uganda*
[3] *Makerere University College of Health Sciences, Mulago Hospital Complex, Kampala, Uganda*

Background: Accurate and early diagnosis of Tuberculosis (TB) is crucial for effective patient management and TB control yet adequate TB diagnosis is still a challenge. Mycobacterial nucleic amplification tests such as polymerase chain reaction (PCR) assays are promising rapid and accurate alternatives that can be done in developing countries. However, the performance of these assays in diagnosis of sputum smear negative pulmonary TB (PTB) has not been widely reviewed.We studied the accuracy of an 'in-house' sputum PCR assay based on detection of IS6110 sequence, in the diagnosis of TB among sputum smear negative PTB suspects.

Methods: A cross sectional study was conducted between September 2007 and February 2008 on the emergency medical ward of Mulago Hospital, Kampala, Uganda. After informed consent, we screened patients aged 13 years and above clinically suspected to have PTB by direct Z-N smear microscopy for acid fast bacilli (AFB). AFB sputum smear negative PTB suspects were recruited consecutively into the study and portions of processed sputum from these study participants were subjected to culture using Lowenstein-Jensen (LJ) media and IS6110-based 'in-house' sputum PCR. Using LJ culture as the gold standard, we analyzed for the diagnostic accuracy of the IS6110-based 'in-house' PCR by computing sensitivity, specificity, positive and negative predictive values.

Results: Overall, 320 PTB suspects were screened, 205 were AFB sputum smear negative and were included in the study. Of these 72/205 (35%) were positive for *Mycobacterium tuberculosis* on LJ culture while 128/205 (62.4%) were positive on the IS6110-based 'inhouse' sputum PCR. Compared to LJ culture, the sensitivity and specificity of the 'in-house' sputum PCR were 75% and 35.9% respectively and the positive and negative predictive values were 39% and 72.4% respectively.

Conclusion: The IS6110-based 'in-house' sputum PCR showed poor sensitivity and specificity in detection of TB among AFB sputum smear negative PTB suspects and therefore may not be adopted as a rapid and accurate alternative to LJ culture. We recommend further evaluation of the IS6110-based 'in-house' sputum PCR in sputum smear negative PTB diagnosis using a more accurate gold standard than LJ culture.

doi:10.1016/j.ijid.2010.02.435

75.036

Amplification of pre-membrane and non-structural region 5 for detection and typification of Dengue virus by real-time PCR using SYBR green I assay

S. Vielma*, M. Odreman, D. Atchley, J. McAvin, G. Comach, C. Torres, G. Aguilera, A. Chiarello, L. Tellez, M. Muñoz

Universidad de Los Andes, Facultad de Medicina, Merida, Merida, Venezuela

Background: Confirmation of Dengue virus [DV] infection by laboratory and serotyping is critical information for patient's management and to applying public health strategies to control the *Aedes aegyptis* vector. Molecular biology methods have largely displaced the classical cell culture techniques reducing the time of reporting. The aim of this study was standardized a Real-Time PCR assay for detection, serotypification and quatification of the DV using SYBR® green I RT-PCR technologies.

Methods: DV serotypes (DV1-4) were selected from the laboratory collection and human serum/blood specimens were collected from febrile patients clinically suspected of DV infection (WHO criteria), during the first three days of clinical symptoms. Total RNA were extracted from the patient samples using a QIAmp®RNA Viral/Blood Mini-Kit (QIAGEN®). Three different universal primers designed to amplified pre-membrane (CprM) and nonstructural protein 5 (NS5) were used during the detection assay. Subsequently, the viral types were determined using the CprM forward primer and a specific reverse primer for each virus serotype [DV1-DV4]. The Reverse Transcription and amplification assays were performed in one step protocol using SYBR® Green-I RT-PCR kit (QIAGEN®) in a 38 cycle process.

Results: All viral types were amplified with the three universal assays. The average CT values for the three region and four-serotypes showed a CprM primer of 26,5; First NS5 region (3NC) 15,9, and finally for Second NS5 region (JMC) 20,9. When the specific reverse primers for DV1-4 were used, only the amplification occurs in the corresponding viral serotype. Validation assay was performed in two hundred-eight serum samples from febrile patients, 33.7% (70/208) of them were positive by RT-PCR. Genotyping showed DV2 (31,1%) as the most frequent genotype, followed by DV1, DV3 and DV4 and a significant difference was observed in viral load in patients with DHF and SSD.

Conclusion: We compare the efficiency of the amplification of three different regions of Dengue Virus using a Universal SYBR® green I RT-PCR assay. Amplification of First NS5 region (3NC) showed the best amplification efficiency in patients and controls. Serotyping assay showed the circulation of DV1-DV4 in our communities.

doi:10.1016/j.ijid.2010.02.436